

COMPOSITION AND LIFE CYCLES OF NECROPHAGOUS FLIES INFESTING
WRAPPED AND UNWRAPPED RABBIT CARCASSES IN JOHOR FOR
FORENSIC APPLICATIONS

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To my beloved family and friends

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ABSTRACT

In forensic cases, corpses are frequently discovered wrapped in some material, probably as a means for disguising, as well as enabling easier handling and preventing evidence transfer. The use of such wrapping materials may affect insect colonization patterns, which in turn causing erroneous estimation of post-mortem interval (PMI). Hence, this present study utilized rabbit carcasses wrapped in a used rug with both sides remained open provided empirical data on species composition and completion of life cycles of necrophagous flies infesting in Johor, Malaysia. Result does not revealed differences in species composition between the wrapped and unwrapped rabbit carcasses. Six necrophagous fly species (*viz.* *Chrysomya megacephala*, *Chrysomya rufifacies*, *Hemipyrellia tagaliana*, *Ophyra chalcogaster*, *Ophyra spinigera* and unidentified Sarcophagidae) were consistently observed in all the decomposing wrapped and unwrapped rabbit carcasses during the three replicate experiments. While completion of life cycle for Sarcophagidae was not observed during the 30 days period of observation, completion of life cycles for the remaining five species i.e. *C. megacephala* (7.66-7.91 days), *C. rufifacies* (8.33-8.50 days), *H. tagaliana* (~14 days), *O. chalcogaster* (~15 days) and *O. spinigera* (~16 days) were observably similar in all the carcasses examined in this present study. Since forensic entomological baseline data for Johor on oviposition and completion of life cycle for these necrophagous flies remain unreported, the results reported here may prove useful for estimating minimum PMI *via* entomological assessment within this region or within the similar biogeoclimatic conditions.

ABSTRAK

Dalam kes forensik, mayat sering ditemui dibungkus menggunakan bahan tertentu dengan tujuan menyembunyikan mayat serta memudahkan pengendalian dan mengelakkan pemindahan bukti. Penggunaan bahan pembungkus berkemungkinan mempengaruhi corak infestasi serangga yang kemudiannya menyebabkan kesalahan dalam menganggar sela-masa kematian (PMI). Justeru, kajian ini menggunakan bangkai arnab yang dibungkus menggunakan ambal terpakai dengan kedua belah hujungnya dibiarkan terbuka menyediakan data empirikal komposisi spesies dan kitar hidup lengkap lalat nekrophagus yang menginfestasi bangkai arnab di Johor, Malaysia. Hasil kajian tidak menunjukkan perbezaan dalam komposisi spesis antara bangkai arnab yang dibungkus dengan yang tidak dibungkus. Enam spesis lalat necrophagous (iaitu *C. megacephala*, *C. rufifacies*, *H. tagaliana*, *O. chalcogaster*, *O. spinigera* dan Sarcophagidae yang tidak dapat dikenalpasti) dapat diperhatikan secara konsisten pada semua bangkai arnab yang dibungkus dan juga tidak berbungkus dalam ketiga-tiga replikat eksperimen. Meskipun kitar hidup lengkap Sarcophagidae tidak diperhatikan sepanjang 30 hari pemerhatian, kitar hidup lengkap lima spesis yang lain i.e. *C. megacephala* (7.66-7.91 hari), *C. rufifacies* (8.33-8.50 hari), *H. tagaliana* (~14 hari), *O. chalcogaster* (~15 hari) dan *O. spinigera* (~16 hari) didapati serupa dalam semua bangkai arnab yang dikaji. Memandangkan, data asas entomologi forensik di Johor berkenaan pengovipositan dan tempoh kitar hidup lengkap untuk lalat nekrofagus belum lagi dilaporkan, hasil kajian ini mungkin berguna dalam menganggar PMI menerusi penilaian entomologi dalam rantau ini atau kawasan yang memiliki biogeoklimatik yang serupa.

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LIST OF ABBREVIATIONS

a.m.	-	<i>ante meridiem</i>
ADD	-	Accumulated degree days
ADH	-	Accumulated degree hours
ANOVA	-	Analysis of Variance
<i>C. megacephala</i>	-	<i>Chrysomya megacephala</i>
<i>C. rufifacies</i>	-	<i>Chrysomya rufifacies</i>
CGS	-	Crow-Glassman Scale
cm	-	Centimetre
CO1	-	Cytochrome oxidase 1
CO11	-	Cytochrome oxidase 2
DNA	-	Deoxyribonucleic acid
e.g.	-	Exempligratia (For example)
etc.	-	Et cetera
<i>H. tagaliana</i>	-	<i>Hemipyrellia tagaliana</i>
i.e.	-	In essence
Inc.	-	Incorporation
IQR	-	Inter Quartile Range
kg	-	Kilogram
KOH	-	Potassium Hydroxide
mm	-	Millimetre
<i>O. chalcogaster</i>	-	<i>Ophyra chalcogaster</i>
<i>O. spinigera</i>	-	<i>Ophyra spinigera</i>
p.m.	-	<i>post meridiem</i>
PMI	-	Post-mortem interval
SD	-	Standard Deviation

- sp - species
- Viz.* - Videlicet (namely)

LIST OF SYMBOLS

%	-	Percentage
°C	-	Degree Celsius
~	-	approximately
±	-	Plus-minus sign
↔	-	Rain throughout the day
Δ	-	Rain in the forenoon
●	-	Rain during the night
◇	-	Rain in the afternoon
‡	-	Intermittent rain
Ø	-	No rain
α	-	Alpha

CHAPTER 1

INTRODUCTION

1.1 Background

Forensic entomology is a subdivision of forensic science (Gennard, 2007) that originates from two Greek words i.e. *entomon* (insects) and *logos* (study) (Hogue, 1993). Forensic entomologist mainly deals with insect evidence recovered from dead bodies for estimating the post-mortem interval (PMI) i.e. the time elapsed between death and the discovery of the body (Eberhardt and Elliots, 2008). It has been indicated that within 72 hours of death, pathological changes such as rigor mortis, algor mortis, and livor mortis would provide reasonably accurate information to forensic pathologist for estimating PMI (Gennard, 2007). However, such pathological changes would no longer provide reliable information for estimating PMI as the duration of discovery exceeded that of 72 hours after death. In such situation, entomological evidence have been reported to provide more reliable estimation of PMI (Gennard, 2007; Mahat and Jayaprakash, 2013).

In general, estimation of PMI using entomological evidence relies largely on the fact that insects are poikilotherms i.e. they are unable to regulate body temperature for sustaining biochemical processes (Higley and Haskell, 2001). Studies further

reveal that the oviposition and developmental patterns of necrophagous insects vary according to variations in biogeoclimatic conditions (Goff, 2009) (e.g. habitat and rainfall) and presence of toxic substances (Mahat *et al.*, 2009; Kelly *et al.*, 2009a), as well as physical condition of the decomposing body (Mahat *et al.*, 2016). Because mitigation of insect development by any factors e.g. biogeoclimatic conditions and presence of drugs and poisons can potentially alter its oviposition and developmental patterns (Goff and Lord, 2010), conducting forensic entomological studies across the varying conditions and habitats appears necessary (Mahat *et al.*, 2014).

It has been indicated that the presence of ammonia-rich compounds and moisture within the decomposing body being a factor that attracts colonization of necrophagous insects, usually within minutes of death (Abd-Rashid *et al.*, 2008b). In view of the categorical roles of insects, Smith (1986) divided them into (a) necrophagous species, (b) predators and parasite of necrophagous species, (c) omnivorous species and (d) adventive species. Being the primary colonizers on corpses and carcasses during the early stage of decomposition, necrophagous species especially Calliphoridae have been responsible for majority of the biomass loss (Anderson, 2010).

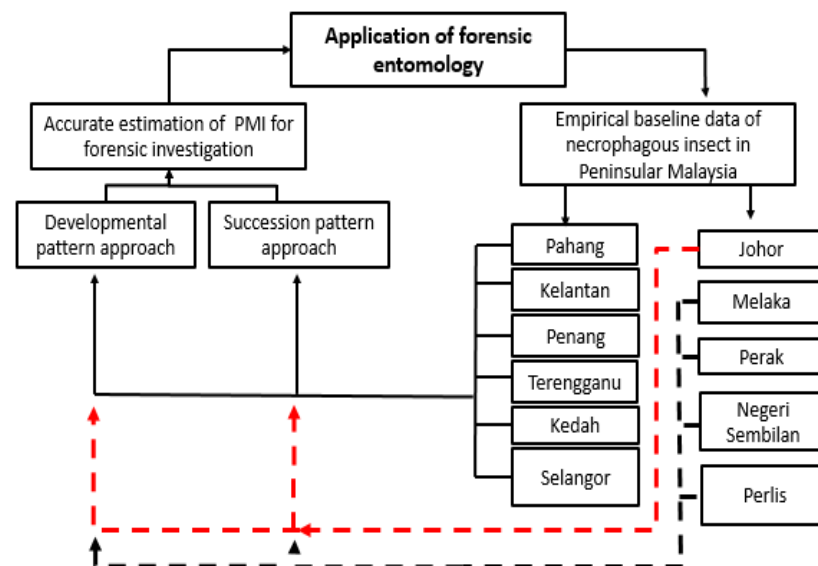
In this context, it is pertinent to indicate that about 500 murder cases are reported yearly in Malaysia (Kosmo, 11 September 2017), and in many cases the bodies were recovered wrapped, probably as a means to cover/hide the bodies. For example, a body of a boy wrapped using a blanket was found hidden in a barrel in a house in Paya Nahu Flat, Sungai Petani, Kedah (Utusan Malaysia, 24 November 2016). In another case example, a body of a boy wrapped in a black plastic bag was found in a drain in Kepong, Selangor (Berita Harian, 21 July 2017). Considering the circumstances surrounding death of such cases, specific forensic entomological studies focussing on the influence of varying wrapping materials/methods merit forensic significance.

In addition to estimation of PMI, entomological evidence especially those from Calliphorid flies have been indicated as useful for determining the presence of antemortem wounds (Eberhardt and Elliotts, 2008), suggesting the possible cause of death (Gennard, 2007; Mahat *et al.*, 2012) and secondary disposal (Campobasso *et al.*, 2001), as well as human identification (Goff, 2009). Interestingly, while species composition and development of necrophagous insects infesting dead bodies and animal carcasses in several states in Malaysia (Mahat and Jayaprakash, 2013) have been reported, the same for other states such as Johor, the southernmost part of Peninsular Malaysia, remains unreported so far.

1.2 Statement of the Problem

Being an integral aspect for forensic investigation, estimation of PMI using entomological evidence can be made by interpreting (a) the life cycle stages of the oldest necrophagous species recovered from the corpse, as well as (b) succession of the different species of insects on the decomposing body (Gennard, 2007). While interpretation of the life cycle for the oldest necrophagous species may provide the minimum PMI estimate (hours to about a week) (Gennard, 2007; Mahat *et al.*, 2009), larger spectrum of estimate (weeks to years) can be provided by interpreting the succession of insects (Anderson, 2010). Because of differences in species composition and duration for completing the life cycle among necrophagous insects due to differences in biogeoclimatic regions have been reported, estimating PMI using the baseline data established in a specific region for another, may prove inappropriate (Anderson, 2010). This aspect triggers the needs for establishing specific entomological baseline data for various biogeoclimatic regions for forensic application.

In forensic cases, corpses are frequently recovered wrapped in some material, probably as a means for disguising, as well as facilitating easier handling and preventing blood stains transfer. In this aspect, accessibility of insects to the body (Voss *et al.*, 2011), as well as the type and extent of wrapping (Anderson, 2010) are factors affecting insect colonization patterns. It has been reported that, wrapping and clothing may initially prevent insect accessibility, however, upon establishment of maggot masses, the clothing and wrapping may provide suitable shelter and microclimate for insects to develop (Kelly *et al.*, 2009b). Interestingly, while general studies on the influence of wrapping on necrophagous insects have been reported, an issue pertaining to the thickness of the material that may have influenced oviposition and life cycles remains unreported, so far. Hence, considering (a) the usefulness of entomological evidence in forensic investigation and (b) because empirical baseline data for composition of necrophagous flies in both wrapped and unwrapped rabbit carcasses in Johor remain unreported, this present study that addresses such important aspects acquires forensic consideration. Putting in forensic perspective within Peninsular Malaysia, the theoretical framework for this present study is presented in Figure 1.1.



Note: Dotted lines represent the incomplete forensic entomology empirical baseline data in Peninsular Malaysia with red ones indicating this present study.

Figure 1.1: Research theoretical framework.

1.3 Objectives and Hypothesis

Considering all the aspects discussed above, this present study was designed:

1. To study the species compositions of necrophagous flies on wrapped and unwrapped rabbit carcasses decomposing in Johor, Malaysia.
2. To compare the oviposition and duration for completing life cycles for necrophagous flies on wrapped and unwrapped rabbit carcasses decomposing in Johor, Malaysia.

It was hypothesized that the duration for completing life cycles for necrophagous flies would be significantly different between wrapped and unwrapped rabbit carcasses.

1.4 Scope of the Study

This present study focused on the composition and completion of life cycles for necrophagous flies infesting wrapped and unwrapped male domestic rabbit (*Oryctolagus cuniculus*) carcasses (1.5-2.0 kg) decomposing during August-October 2016 within the Universiti Teknologi Malaysia (UTM) Johor Bahru Campus. While the wrapped carcasses were wrapped in a layer of used rug (length: 43cm, width: 32cm; thickness: 0.8cm) with both ends remained open, unwrapped carcasses were left to decompose on the ground without wrapping. Following the entomological observation method prescribed by Mahat *et al.* (2016), presence of oviposition, larvae, pre-pupae, pupae and emergence of teneral, as well as ambient and carcass temperatures, total daily rainfall and humidity were recorded.

1.5 Significance of the Study

The data gathered in this study elucidated for the first time, the species composition and completion of life cycles for necrophagous flies infesting wrapped and unwrapped rabbit carcasses decomposing in Johor, Malaysia. In view of its practical values in forensic investigations of unnatural deaths, this present study would pave the way for completing the forensic entomological baseline data for Malaysia. The data can be particularly useful for estimating the time interval since death for human bodies as well as wildlife animals wrapped with similar wrapping materials/methods for forensic investigations in Malaysia and other countries with similar biogeoclimatic conditions.

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